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SOME CONTRIBUTIONS OF ELECTRON MICROSCOPY TO PROBLEMS IN PATHOLOGY

*The Twenty-Fourth Middleton Goldsmith Lecture**

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IT HAS frequently been the custom for a Middleton Goldsmith lecturer to employ the podium for the hour placed at his discretion in reviewing past achievements in the field of pathology. The historical retrospective survey is a safe subject and, although demanding of some scholarship, discretion, tolerance and, perhaps, even wit, it is certain to arouse no great disagreement from the listeners. The recital of glorious victories won by science in the past leaves everyone content and edified, and certainly not disputatious. He who elects to discuss current advances, however, invites criticism if not downright disbelief. The rare lecturer who chooses to develop a thesis in support of new pathways to be followed or new instruments to be employed in the pursuit of

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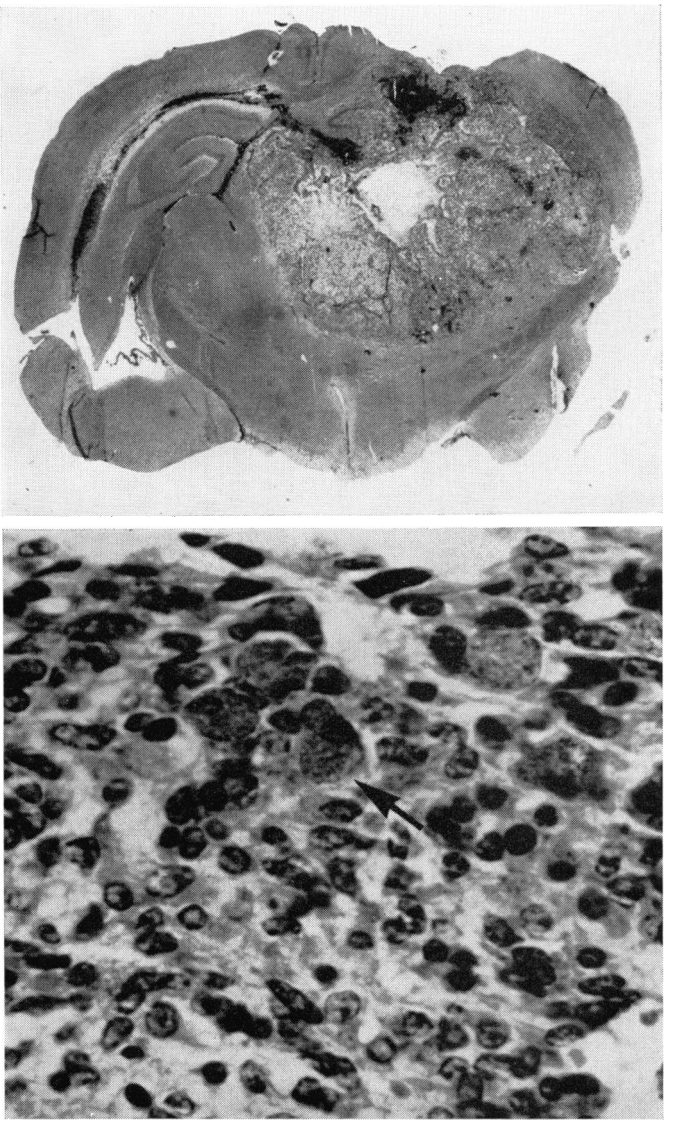

Fig. 1. (Top) Photomicrograph of sagittal section of mouse brain showing large glioma surrounding central cavity which represents site of dissolved methylcholanthrene pellet. Hematoxylin-eosin stain; x 9.5.

Fig. 2. (Bottom) Incipient glial proliferation at site of implantation of chemical carcinogen. Pigment of methylcholanthrene can be seen in a number of gliocytes. Hematoxylin-eosin stain; x 680.

knowledge begs for disinterest and risks being left to talk to himself.

In selecting the subject of this particular Goldsmith lecture the essayist was fully aware of these hazards. It was not expediency which dictated the selection, but rather the conviction that the electron microscope is already a valuable aid in the solutions of problems in pathology which can be approached in no other way, and that this instrument with its future modifications will continue to be of considerable value in the field of biology in the forseeable future.

There are pathologists who question the usefulness of the instrument on the grounds of high initial cost and subsequent maintenance, space requirements in installation and operation, complexity of design necessitating long hours of training in its use and recourse to factory-trained specialists for repairs, and finally the necessarily complicated techniques in processing tissue for examination. To all these disadvantages there must be added, of course, the enormous difficulty in interpretation of the electron micrographs. We simply haven't as yet anything like complete knowledge of the normal ultrastructure of tissues and cells, let alone the ultrastructural modifications in their infinite varieties which occur as the result of injury to these tissues and cells.

It should be acknowledged at the outset that electron microscopy will certainly not solve all, probably not most, and even perhaps not many, problems in biology. But for what this instrument is specifically designed to do, namely, to reveal the ultrastructure of things, the serious morphologist has no substitute. The electron microscopist, in small measure perhaps, is today on the same threshold where once stood Galileo as stated by I. Bernard Cohen, professor of the history of science at Harvard University, who wrote that the philosophers and men of science of that era "had no source of information beyond what the unaided eye could see; Galileo had a telescope".[1]

In the presentation which follows, an attempt will be made to show to what degree electron microscopy has been instrumental in elucidating pathogenetic factors and morphologic details in several conditions which have remained unresolved until the present. These conditions have engaged the interest of this investigator and his colleagues for a number of years; their study has also been actively pursued by many investigators in other laboratories. The experimental approach to their solution, utilizing light microscopy, reached an impasse which only now holds promise of solution.

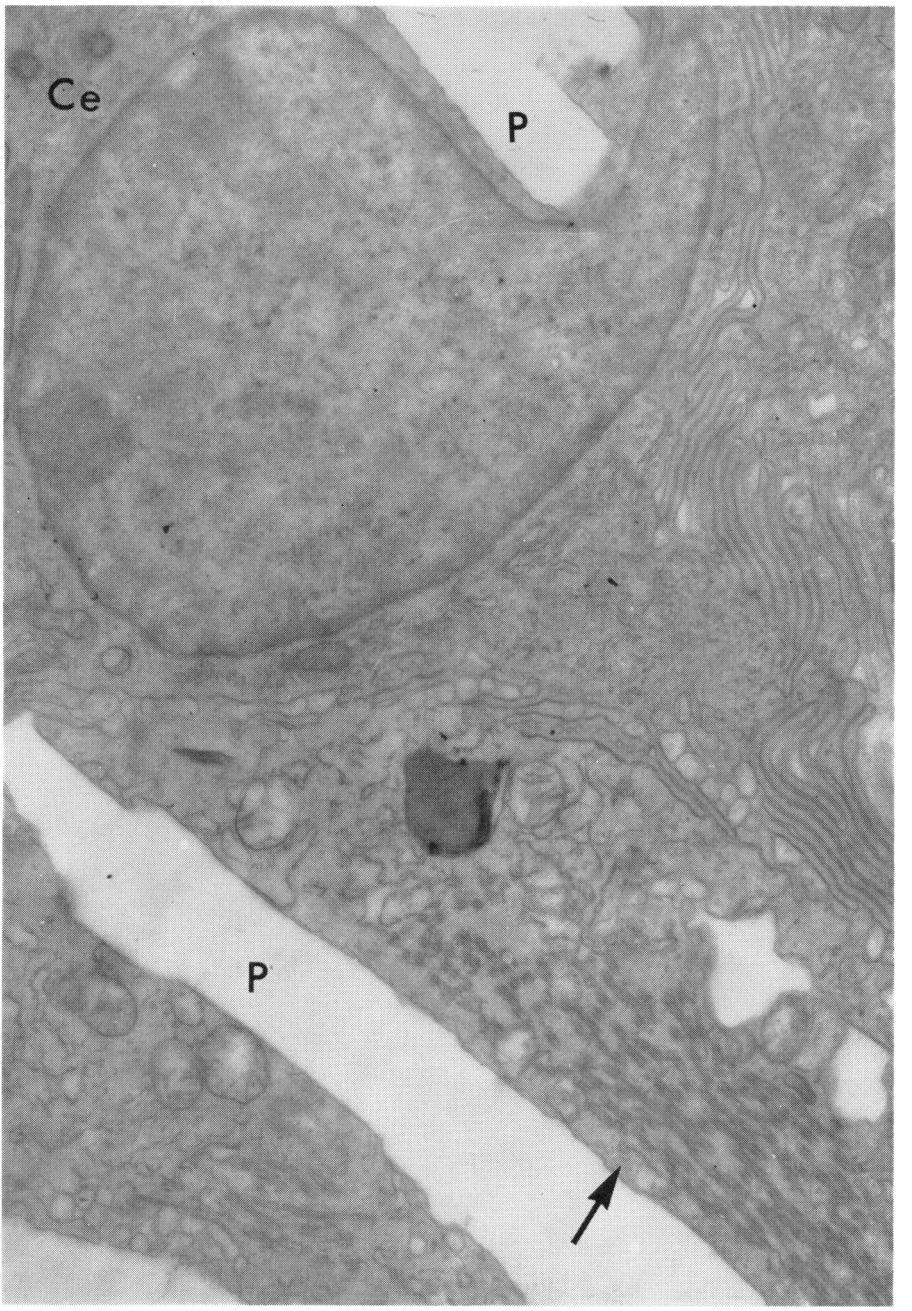

Fig. 3. Electron micrograph of subependymal zone of lateral ventricle two months following implantation of pellet of dibenzanthracene. Filamentous rods in large numbers and variously directed lie within cytoplasm. These particles are present near crystal spaces left by dissolved carcinogen and lined by double membrane. Note two centrioles (Ce) in upper left field. Stained with lead hydroxide; x 23,000.

Chemical Carcinogenesis

For more than twenty years, on and off, my co-workers and I have been engaged in a study of brain tumor production with a variety of chemical carcinogens in a number of different inbred strains of mice. Already in the first report which detailed these experiments[2] it was shown that after a lapse of nearly a year almost 50 per cent of the animals developed gliomas at the sites of chemical implantation (Figure 1). It was, however, also shown that pellets of the carcinogens were found embedded in the normal cerebral tissues of mice which failed to develop tumors. Yet the pellets removed from both the positive and negative tumor groups were equally effective in producing brain tumors later when implanted in other mice. This experience indicated that other factors in addition to the carcinogens were important in tumor production.

In a later contribution[3] it was shown that incipient glial proliferation occurred at the sites of chemical implantation. Light microscopy disclosed the fact that carcinogenic pigment was found within these proliferating gliocytes (Figure 2), but this investigative tool was powerless to reveal what went on within the pigmented cells or, indeed, how the pigment came to occupy its intracellular position. The step-by-step unraveling of these events was left to my associate, Dr. Fusahiro Ikuta, who undertook their electron microscopic study and documented them in conclusive detail. Utilizing dibenzanthracene as the carcinogenic agent, Dr. Ikuta found the spaces of the dissolved crystalline chemical within two months after implantation (Figure 3). At this stage the crystals were lined by double membranes, indicating their extracellular position. But already within the cells there were present many filamentous rods strewn in different directions.

Four and one-half months after carcinogen implantation, the electron micrographs revealed both filamentous rods and spheres within the cellular cytoplasm (Figure 4). The particles had distinctive shapes, size and structure. At a magnification of 33,000 diameters, empty spaces of carcinogen left behind when the chemical was dissolved out during embedding of the tissue were found intracellularly. Under higher magnification (x 100,000) the particles showed continuity of their outer shells (Figure 5). There was also a suggestion that a continuity existed between endoplasmic reticulum and the outer shell of some particles.

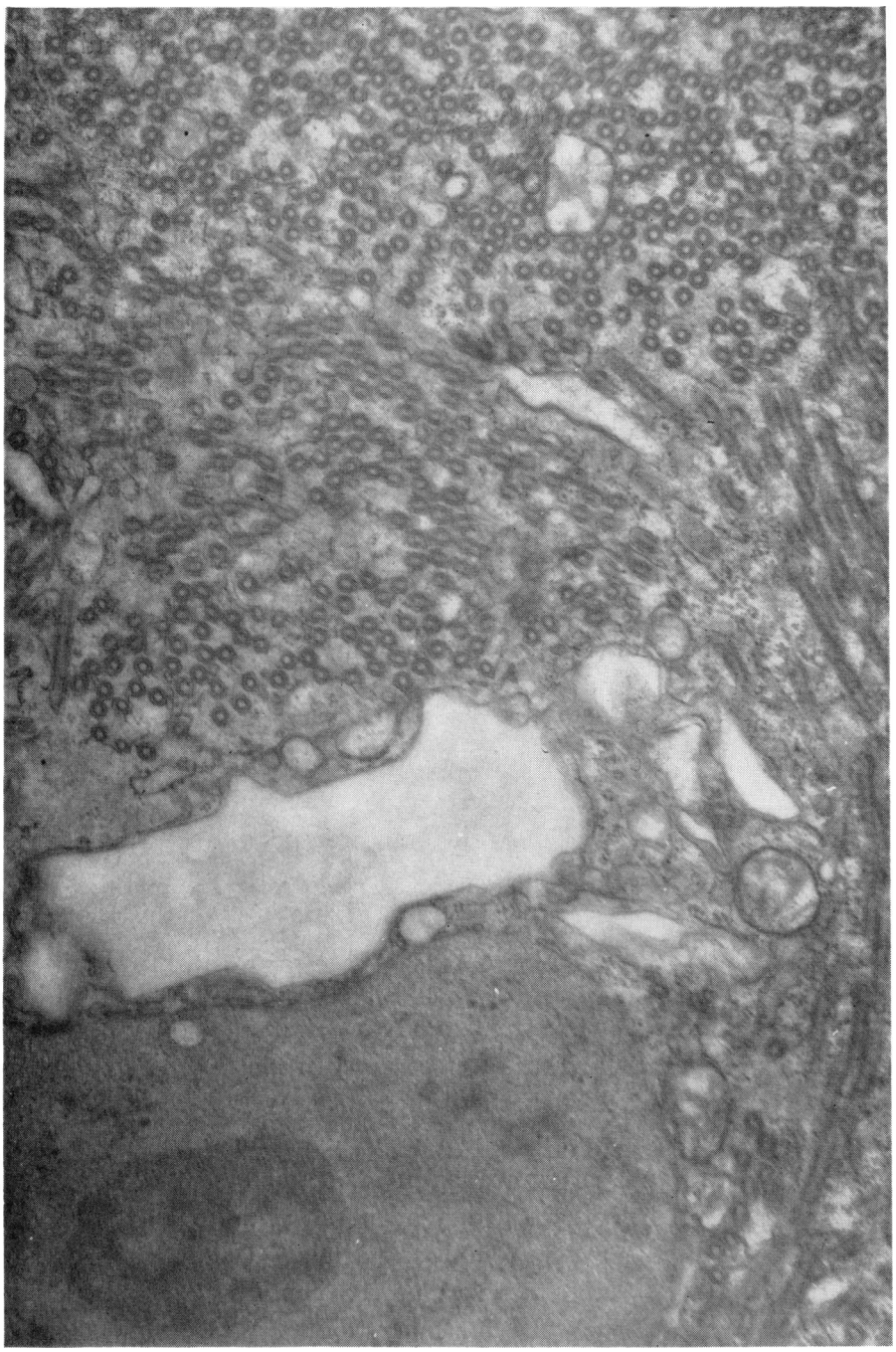

Fig. 4. Filamentous rods and spheres within cellular cytoplasm from specimen removed from right parietal lobe into which was implanted a pellet of carcinogen four and one-half months previously. An empty space formerly occupied by carcinogen is seen near the nucleus. Stained with lead hydroxide; x 33,100.

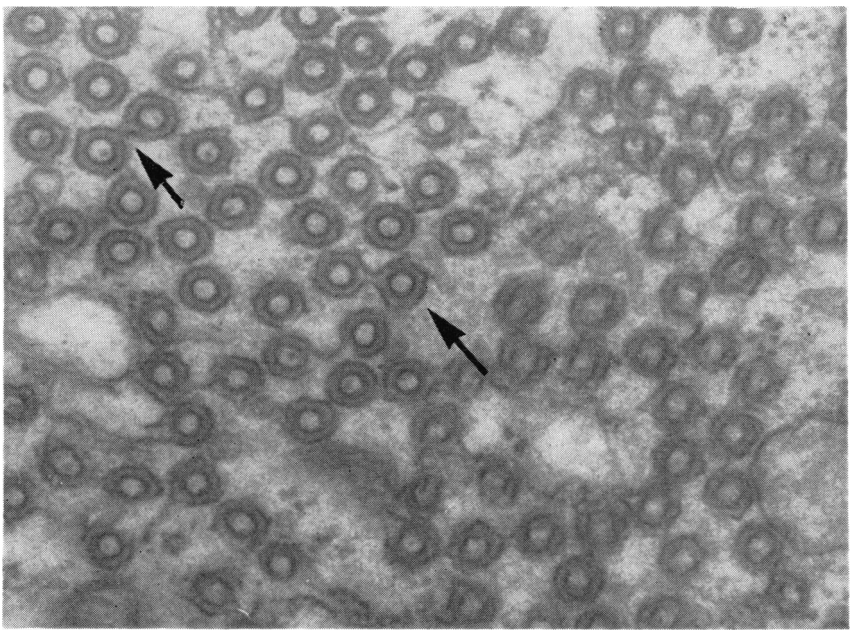

Fig. 5. Upper portion of Figure 4 under higher magnification to show continuity of outer shells of particles (indicated by arrows). There is a suggestion also of continuity between endoplasmic reticulum and the outer shell. Stained with lead hydroxide; x 100,000.

The spheres had the appearance of doughnuts due to dense inner and outer shells (Figure 6). The diameter of the latter was 770 Å, and of the inner shell 380 Å. Within the central cavity there was an occasional dense, relatively large core which measured 100 to 150 Å. The inner shell seemed to be formed by 10 to 13 fine, dense granules which were arranged around the axial cavity. In the filamentous forms of the particles, the dense granules were spaced approximately 20 per 180 mμ. The ends of the filaments opened freely into the cytoplasmic matrix.

Other chemical carcinogens such as methylcholanthrene and benzpyrene yielded similar results. Dr. Ikuta found that the spaces left behind by the dissolved-out methylcholanthrene were lined by walls having a distinctive "saw-tooth" appearance (Figure 7). These cytoplasmic extensions indicated the mechanism whereby the carcinogen was phagocytosed. The tips of the "saw teeth" were sometimes found fused around the crystals of hydrocarbon, and clear spaces representing dissolved-out chemical were present well within the cellular cytoplasm.

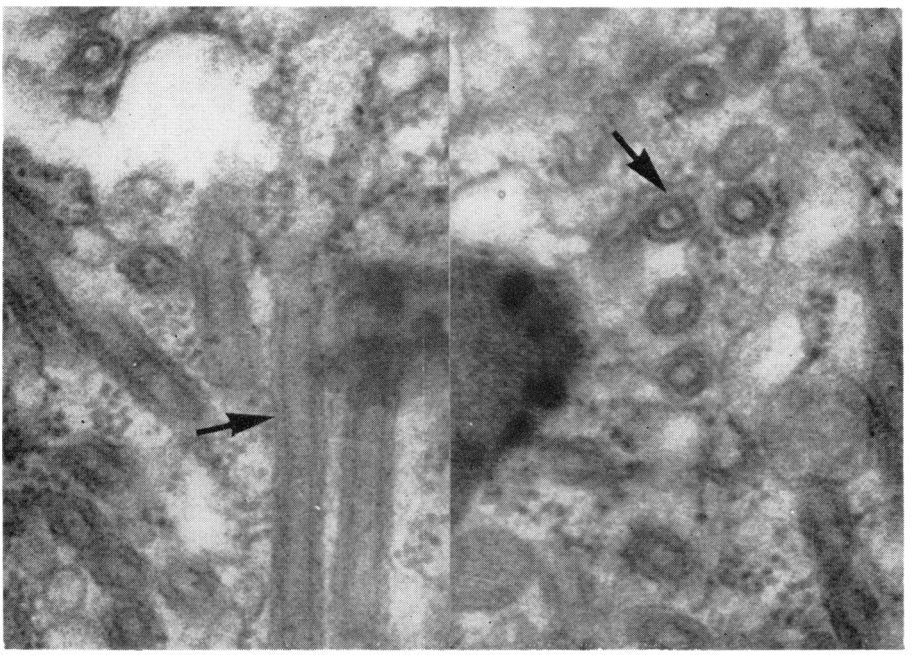

Fig. 6. Fine structure of both filamentous and spherical particles in specimen from right frontal pole following implantation of pellet of carcinogen seven months previously. Note "doughnut" appearance of spheres formed by dense outer and inner shells. The diameter of the outer shell is 770Å and of the inner, 380Å. Within the central hole or cavity there is an occasional dense, relatively large core which measures 100 to 150Å. The inner shell seems to be formed by 15 to 13 fine, dense granules which are arranged around the axial cavity. In the filaments these granules are spaced approximately 20 per 180 mμ. The ends of the filaments open freely into the cytoplasmic matrix. Stained with lead hydroxide; x 96,800.

Spheres and cylindrical rods were present in the projections of the cytoplasmic matrix into the clear spaces remaining at the sites of dissolved carcinogen (Figure 8). These particles in general had the same morphologic characteristics already described for those associated with dibenzanthracene. Occasionally, however, they presented unusual variations in appearance. Some particles seemed to lie within the endoplasmic reticulum (Figure 9). They lacked outer shells of their own, the endoplasmic reticulum apparently subserving this function. Also noted were adjacent rods whose outer shells were continuous; two rods frequently shared one continuous outer shell.

Following the intracerebral implantation of benzpyrene, two months or more elapsed during which no intracytoplasmic particles were in

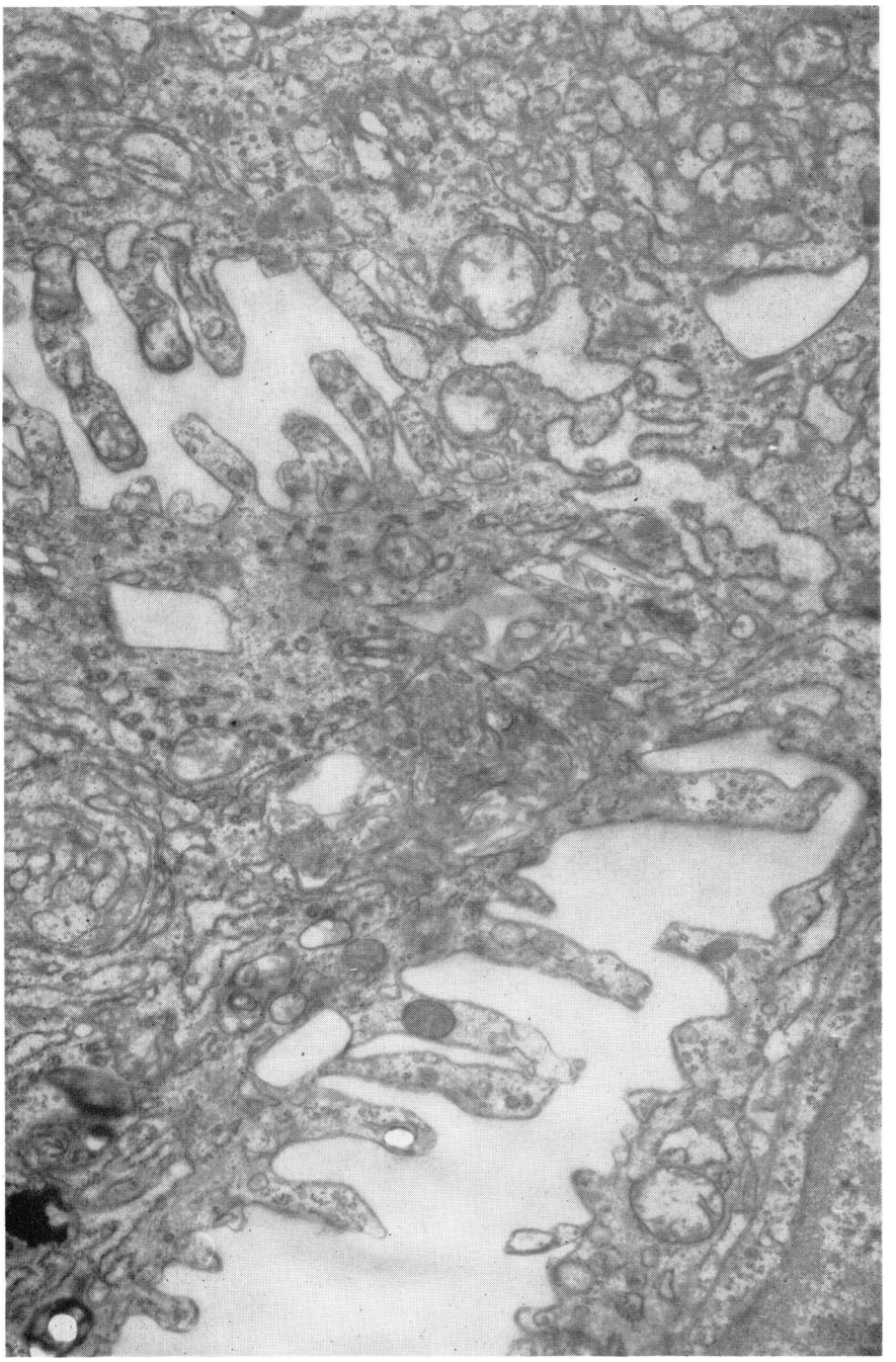

Fig. 7. Electron micrograph showing empty spaces left by dissolved-out methylcholanthrene crystals. Note "saw-tooth" appearance of walls lining the spaces; this illustrates the mechanism of phagocytosis of the carcinogen, as seen by the presence of clear spaces within the cellular cytoplasm. Stained with lead hydroxide; x 20,000.

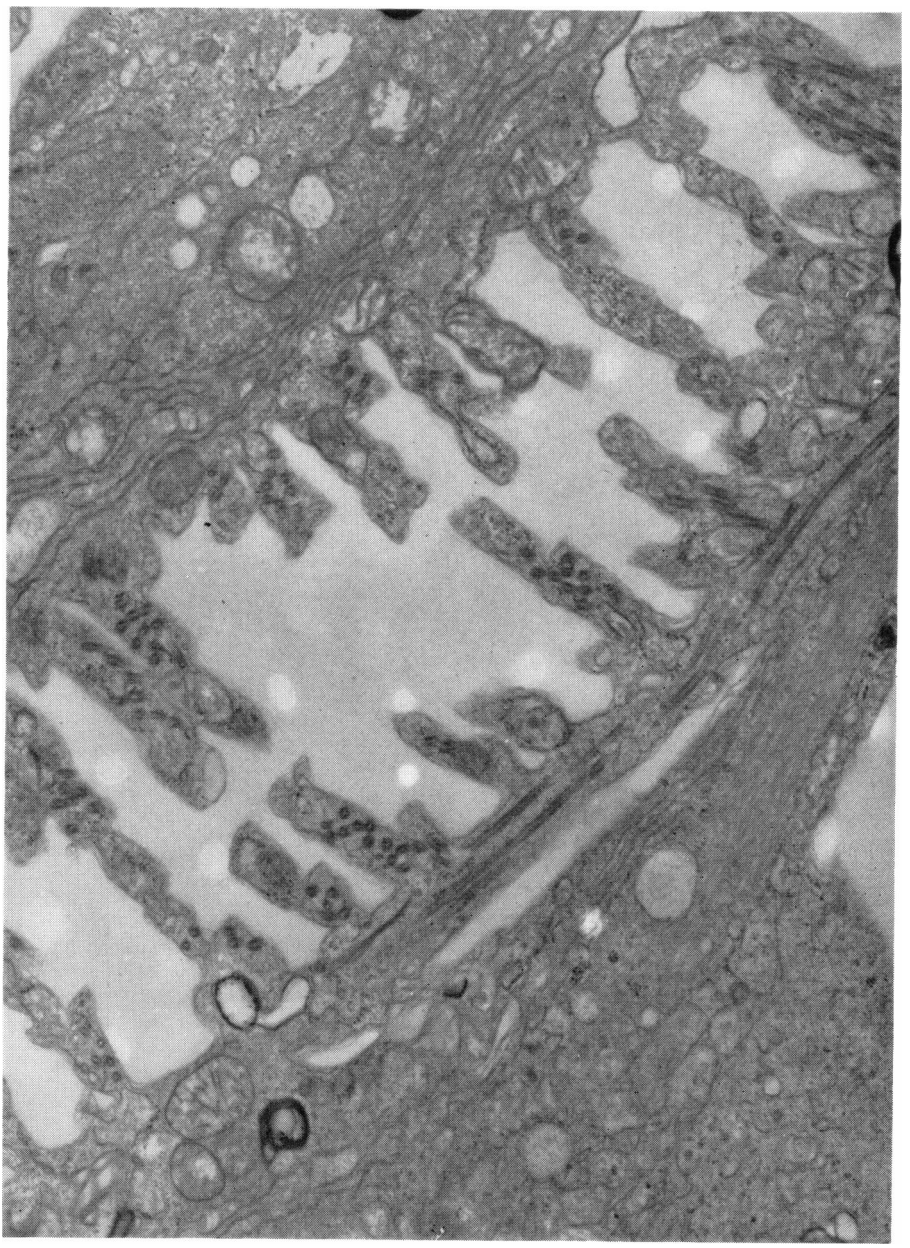

Fig. 8. Spheres and cylindrical rods are present in the projections of cytoplasmic matrix into the clear space remaining at the site of dissolved carcinogen. Stained with lead hydroxide; x 21,500.

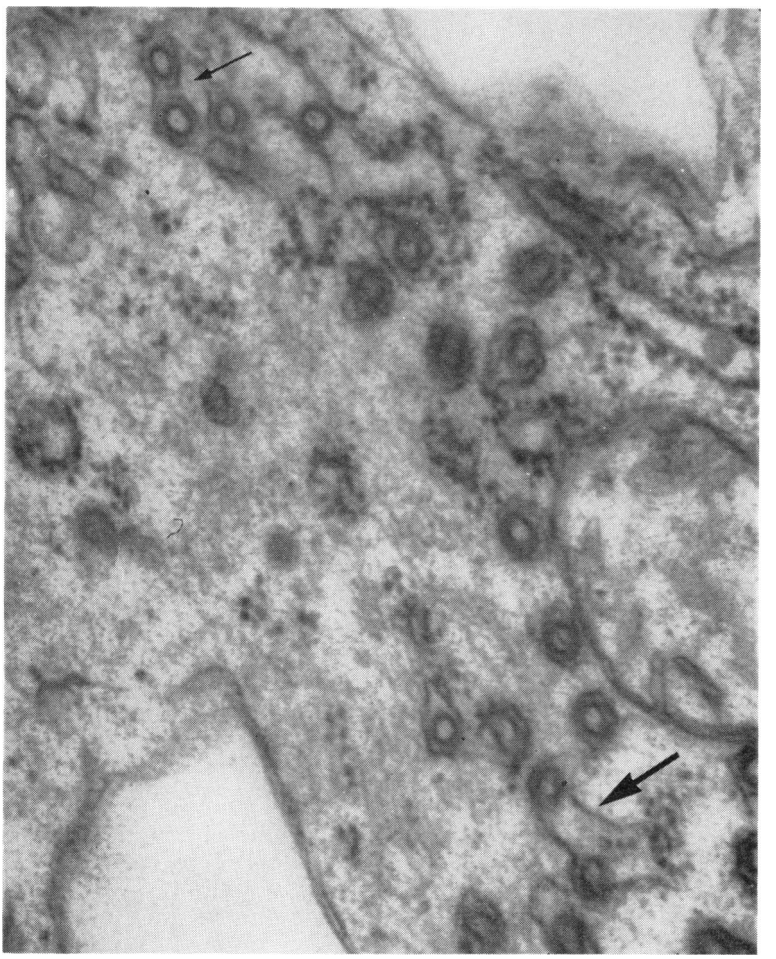

Fig. 9. Unusual appearance of particles, some of which seem to lie within the endoplasmic reticulum (arrows). These rods seem to lack outer shells of their own, but the endoplasmic reticulum appears to serve this function. Note also the continuity of the outer shell around two adjacent rods. Stained with lead hydroxide; x 100,000.

evidence. Then gradually both spherical and filamentous particles made their appearance (Figure 10). Again there was seen phagocytosis of the carcinogen which occurred just prior to, or simultaneous with, the development of the characteristic intracytoplasmic particles.

These filamentous and spherical particles were thus found within reactive cells induced by the intracerebral implantation of a variety of chemical compounds. They were present within the cells during the

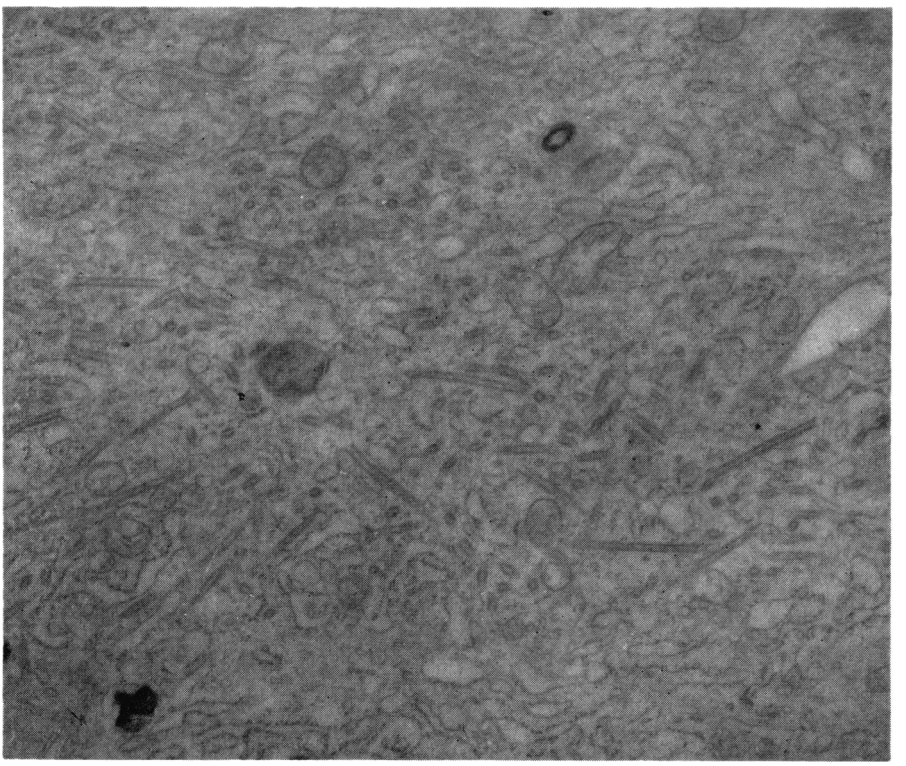

Fig. 10. Numerous spherical and filamentous particles in the cytoplasmic matrix following the intracerebral implantation of benzpyrene two months previously. Note the empty space left by the dissolved carcinogen. Stained with lead hydroxide; x 22,000.

period of the so-called precancerous stage and in most of the animals with implanted pellets of carcinogen. They were not found within the glial cells which formed gliogenous neoplasms; at a point when the proliferating glial cells became malignant these particles seemed to disappear. What their fate is in tumor production still remains to be determined.

Now it is pertinent to inquire into the chemical nature of these particles. Blocks of brain tissue from sites of chemical implantation were fixed in neutral formalin and some were then incubated in solutions of ribonuclease, others in deoxyribonuclease, trypsin or pepsin for different lengths of time at 37°C. Following osmium tetroxide fixation, this material was prepared for electron microscopic study. Control specimens which had been incubated for 70 minutes in Veronal

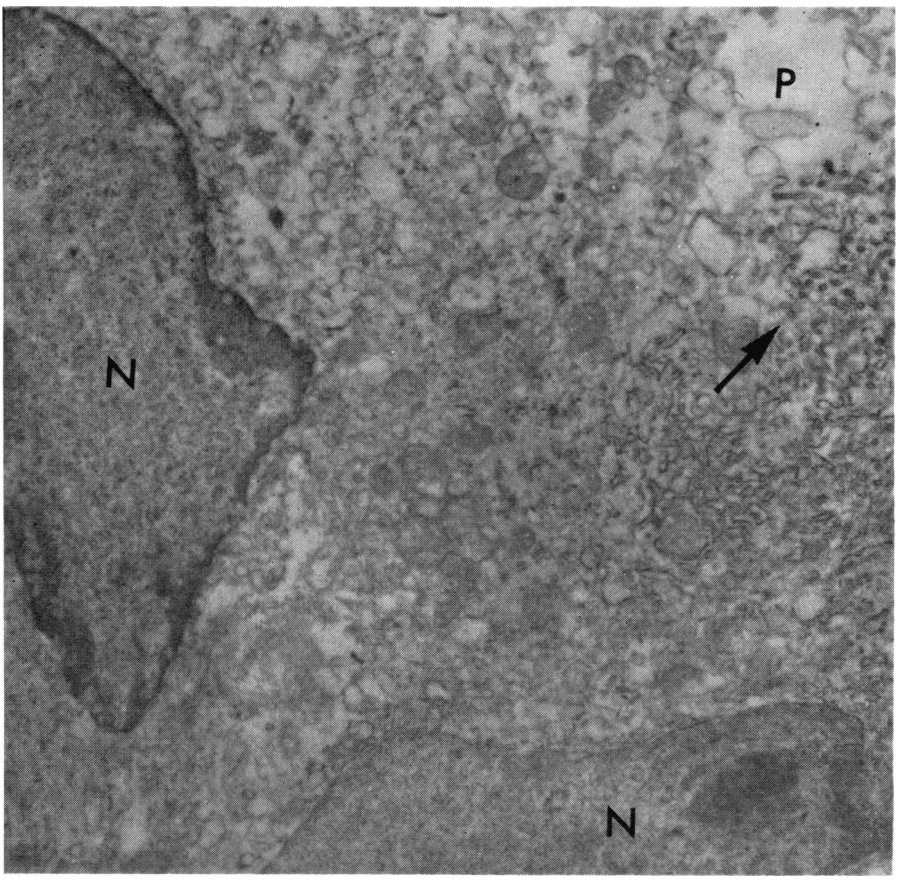

Fig. 11. Electron micrograph from specimen obtained three months after dibenzanthracene implantation. The control block was fixed in neutral formalin, then incubated for 70 minutes in Veronal acetate buffered with magnesium sulphate. The nuclear chromatin (N) in the reactive cell is still electron-dense, and dense spherical particles (arrow) are also present near an empty space (P) left by a dissolved carcinogen crystal. Ribosomes, smaller than the particles, are also present and dense. Stained with lead; x 18,000.

acetate buffered with magnesium sulphate still showed the normal nuclear electron density of the reactive cells (Figure 11). The intracytoplasmic spherical particles were still preserved and were found near the empty spaces left behind by the dissolved carcinogen crystals. Ribosomes were also present. When the tissue was incubated for 70 minutes in a solution of deoxyribonuclease (DNAse) in Veronal acetate buffered with magnesium sulphate, the nuclear chromatin in the reactive cell was nearly completely digested, but the ribosomes and the

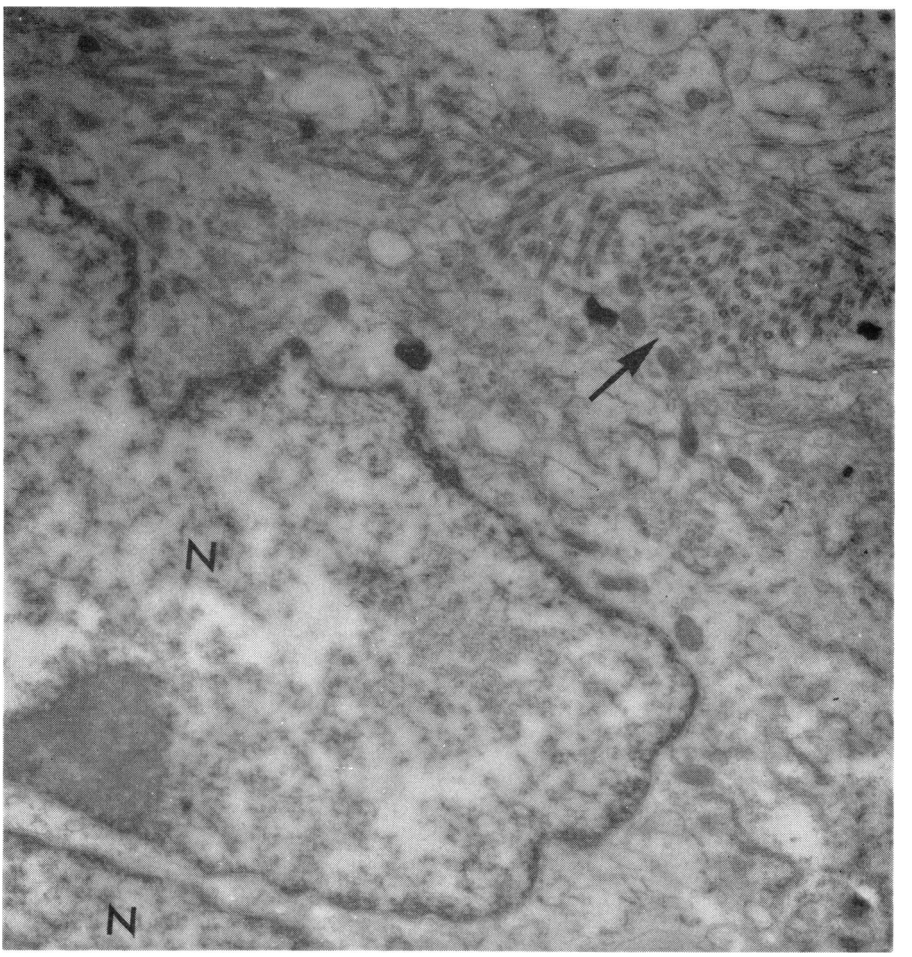

Fig. 12. Nuclear chromatin (N) of the reactive cell is nearly completely digested in tissue incubated 70 minutes in a solution of DNAse in Veronal acetate buffer with magnesium sulphate. Ribosomes and particles (arrow) of normal density are unaffected. Stained with lead; x 18,000.

particles were unaffected (Figure 12). Under higher magnification both ribosomes and central cores were found well preserved both in longitudinal and transverse views of the particles (Figures 13A and B). Incubating the tissue for 40 minutes in a solution of ribonuclease [RNAse], on the other hand, produced almost complete digestion of both the axial cores of the particles and the cytoplasmic ribosomes (Figures 14A and B). Complete disappearance of these structures occurred after

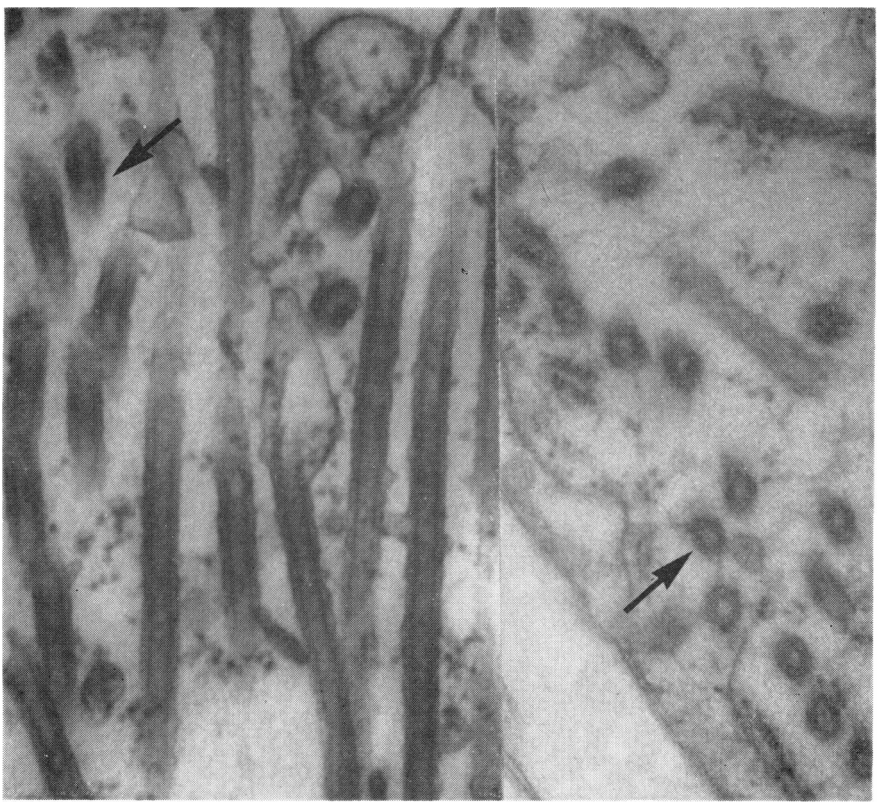

Fig. 13A Fig. 13B

Fig. 13A and B. The axial cavities of the particles, both in longitudinal and transverse section contain electron-dense cores (arrows). The ribosomes are also well preserved and electron-dense, as are the inner shells of the particles. Tissue treated with DNAse. Stained with lead; x 100,000.

60-minute incubation in RNAse. The inner shells of the filamentous rods also showed advanced digestion. Tissue incubated for 20 minutes in a solution of trypsin did not suffer any digestion of ribosomes, axial cores, or nuclear structures, but there was considerable digestion of the structure between the inner and outer shells. Fifteen- to 20-minute incubation of tissue in a solution of pepsin caused such destruction as to invalidate any interpretation.

From these findings it may be concluded with some measure of safety that the axial cores of the filamentous rods contain RNA but not DNA. This is also true of the fine granules which form the inner shells

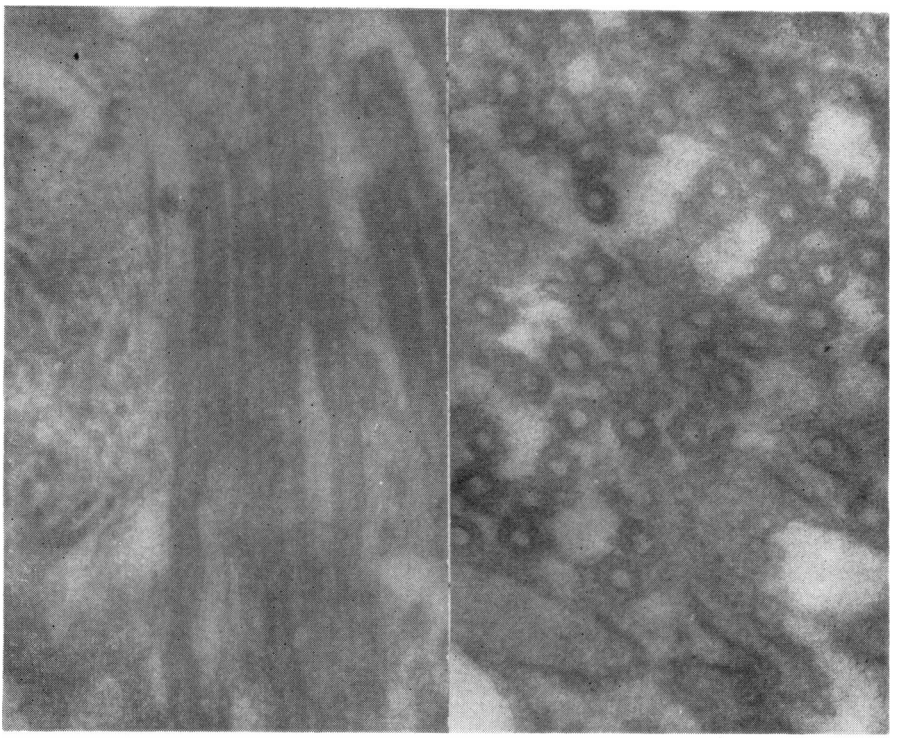

Fig. 14A Fig. 14B

Fig. 14A and B. Electron micrograph of tissue incubated for 40 minutes in a solution of RNAse. Longitudinal and transverse sections of particles reveal an absence of dense axial cores. Also absent are ribosomes. Stained with lead; x 100,000.

of the particles. Further, trypsin digestion of the structure between the inner and outer shells indicates its protein nature. The chemical composition of these mysterious particles is at least not incompatible with their being viruses. There is, in addition, rather strong morphologic support for such a concept. This support comes from Bernhard's study of tumor viruses[4] and from Dalton's study of the Moloney agent in mouse leukemia.[5] This agent, which is remarkably similar to our carcinogen-induced particle, also has a double wall with continuity of the external membrane around two adjacent particles. It, too, is a filamentous hollow rod with an electron-dense central core. In size, however, it is smaller than our particle.

Much, of course, remains to be done in the further identification of this chemically induced particle. Its precise role in experimental glioma production is still to be determined. Why this intracytoplasmic

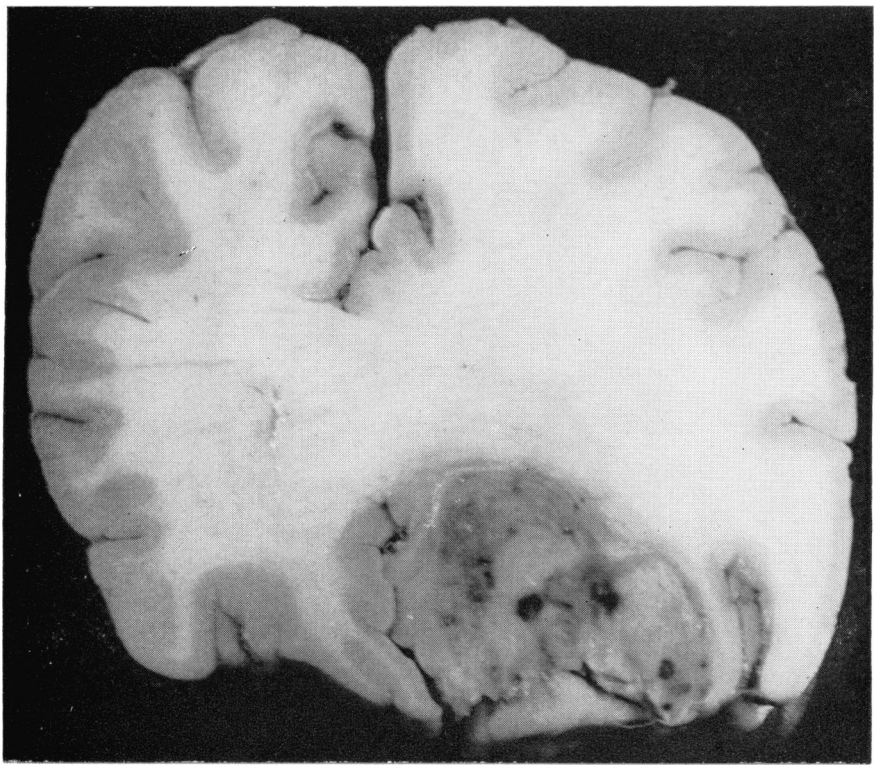

Fig. 15. Glioblastoma multiforme involving right medial orbital gyrus. Severe swelling of centrum semiovale with flattening of gyri and herniation of cingulate gyrus. The swollen hemisphere was firm and relatively dry.

structure first appears after some weeks of exposure of the animal brain to a chemical carcinogen, persisting for some months during the precancerous period, and then mysteriously disappears as the glial cells proliferate in sufficient numbers to form a tumor, is still unknown. It is yet to be shown that cell-free suspensions of these particles can themselves produce gliomas. But considerable progress has been made in the study of cerebral tumorogenesis with the aid of the electron microscope after nearly twenty years of frustration.

Cerebral Swelling and Edema

There is yet another problem of considerable importance which has occupied the attention of pathologists for over fifty years without a solution and which is only now being resolved on the ultrastructural level. This is the problem of cerebral swelling versus cerebral edema.

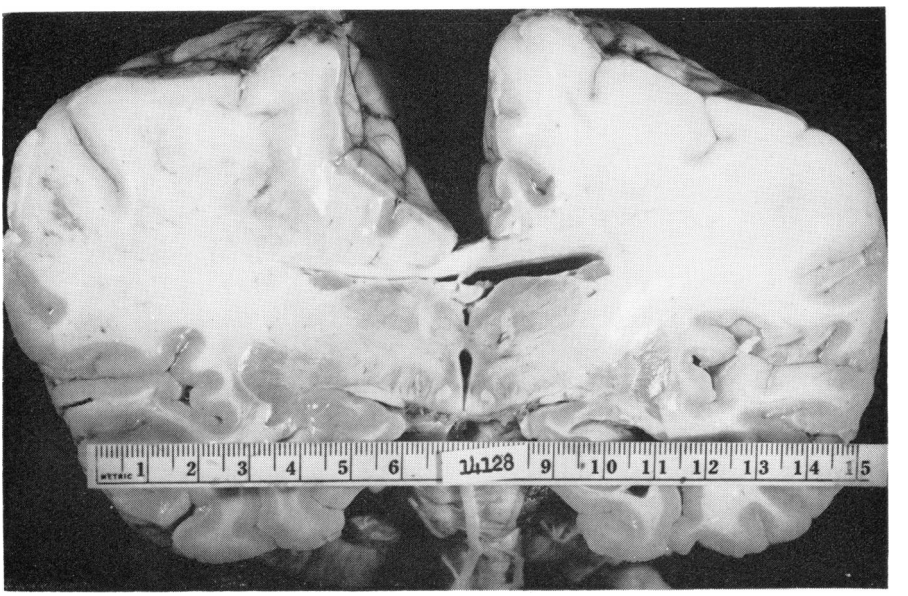

Fig. 16. Swollen, wet brain of soft consistency whose cut surfaces dripped fluid. Patient had extracranial malignant lymphoma with anemia and low plasma proteins.

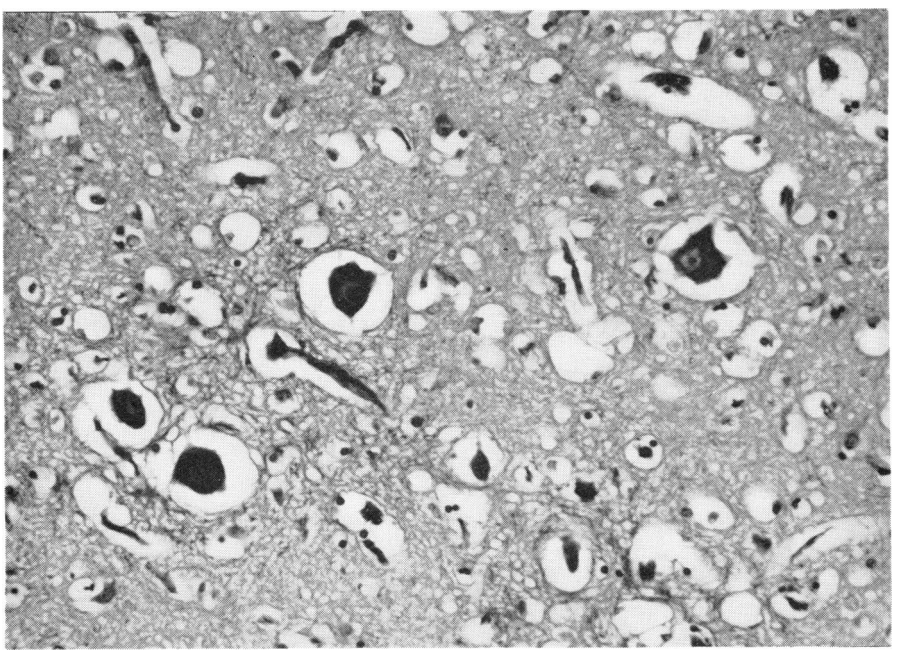

Fig. 17. Perivascular and pericellular fluid accumulation in cerebral cortex of patient who died in status epilepticus. Luxol-fast blue stain; x 250.

Most pathologists are familiar with the enlargements of cerebral hemispheres, affecting mainly the central white matter, which are the seats of neoplasms, both primary and metastatic; such swellings are often vastly out of proportion to the size of the offending tumor (Figure 15). Similar firm and dry swollen brains have been noted in patients who succumbed to epileptic and paralytic attacks, and they have been seen in some patients with uremia and various infections. By contrast, there are brains which are also increased in volume but which are soft and water-logged, free fluid dripping from the cut surfaces (Figure 16). Such brains are frequently found in fatal cases of acute alcoholic intoxication, in severe head injury, and with such poisoning as carbon monoxide, phosphorus and arsenic. They are also found in consequence of serious disturbances in intracranial hemodynamics, such as may occur in chronic congestive heart disease.

To the firm, dry brain of increased volume, the term cerebral swelling has been applied, whereas cerebral edema connotes the enlarged, soft wet brain. Much has been written on the differential features of these two states. In general, the German school of pathologists represented by Reichardt[6] and Zülch[7, 8] hold that both macroscopically and pathogenetically these are separate and distinct conditions. Most other pathologists hold with the view expressed by Greenfield *et al.*[9] who state: "In cases of trauma, tumor or abscess some authors consider that brain swelling and brain oedema can both be present at the same time in the same brain; and there is a further subdivision of opinion as to whether, in such cases, they can be separated microscopically, and whether cerebral edema is but a more severe degree of the same process which may give rise to cerebral swelling."

Conventional microscopic study of these conditions has contributed little to their understanding. Often the very process of preparation of the tissue for light microscopy, necessitating its dehydration preceding embedding for sectioning, removes what fluid accumulations there are as the basis for either condition. When traces of abnormal fluid accumulations survive the drastic artifacts of tissue preparation for histologic examination, no sharp differences are noted between cerebral edema and swelling. What is even more important is that little evidence survives to indicate the circumstances surrounding these abnormal intracerebral fluid accumulations. In the cortical and central gray matter, evidence of excess fluid accumulation may sometimes be seen in cases

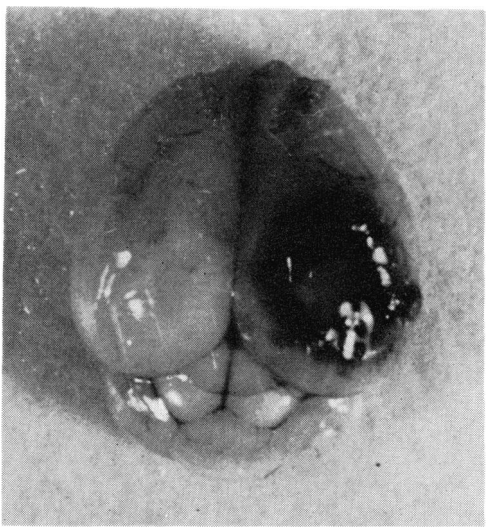

Fig. 18. Swollen right cerebral hemisphere of mouse following cold injury to cortex. Note staining with trypan blue indicating increased permeability of the blood-brain barrier.

of both swelling and edema in the form of clear paraneuronal and paraglyocytic spaces (Figure 17). There is also widening of the Virchow-Robin spaces. Both neuronal and glial cells appear shrunken and compressed. What the condition of the intercellular stroma or neuropil is in regard to its fluid content it is impossible to judge. In the subcortical white matter and centrum semiovale, the perivascular spaces are widened and for variable distances around the vessels the myelinated fibers are separated and loosened. This may be due to abnormal fluid accumulation, but the appearance somewhat resembles patchy demyelination save for the fact that there is neither a macrophagic response nor sudanophilic myelin breakdown.

This was the approximate state of our knowledge regarding this important subject up to 1959 when my colleagues, Drs. Torack and Terry, and I undertook to study the fine structure of cerebral fluid accumulation secondary to cold injury to the brain.[10] Recent investigations of the normal central nervous system by means of electron microscopy had revealed that the cellular elements of the brain apparently fitted together so closely that the extracellular space was probably no greater than two per cent.[11-13] It had also been shown by Klatzo,

Piraux and Laskowski that localized cerebral edema followed cold injury to the cerebral cortex.[14] In our experiment, carbon dioxide ice was applied to the cerebra of young, anesthetized mice and rats for a period of 30 seconds. A solution of trypan blue was injected subcutaneously into six animals in order to demonstrate the passage of the vital dye across the blood-brain barrier into the injured cerebral tissue. In each instance the injured brain became swollen and herniated through the craniotomy wound (Figure 18). Electron microscopic study of the injured cortical gray matter in these animals confirmed the fact that the intercellular space is of the order of 10 to 20 mμ in width. The localized cerebral swelling was manifested by enlargement of the cytoplasmic volume of the glia with clear cytoplasm which Farquhar,[11] Schultz,[12] De Robertis,[15] and their co-workers consider as astrocytes, but which Luse[16] and Palay[17] believe to be oligodendrocytes. The intercellular space was not affected by the swelling.

In view of the strict localization of the fluid within glial cytoplasm, it seemed desirable to study other types of cerebral fluid accumulation. There was, of course, the possibility that different causative agents, different rates of onset and different degrees of severity altered the location of the fluid. The next experiment, therefore, was undertaken with triethyl tin sulfate and triethyl tin hydroxide as constituents of the diet in C_3H mice.[18] The alkyl tin compounds in small amounts (12 to 32 parts per million) in the food of these animals caused diffuse swelling of the brain. Whereas the light microscope seemed to reveal an excess of fluid in the interstitial tissue, the electron microscope disclosed severe swelling of the clear glial cells without an increase in size of the minute intercellular space. It should be stated, however, that the study inadvertently was limited to an examination of the cortical and central gray matter and did not include the subcortical white matter. In this severe form of swelling, numerous glial cells were found whose cell membranes were ruptured. Human cerebral tissue, from the swollen zones surrounding gliomas as well as metastatic carcinoma, was compared with this experimental material and it, too, showed an increase in the cytoplasmic volume of the clear glial cells without an enlargement of the intercellular space. Luse and Harris[19] seemed to confirm these experimental results in rabbits which they injected with hypotonic solutions. This treatment also caused only glial swelling in the neuropil.

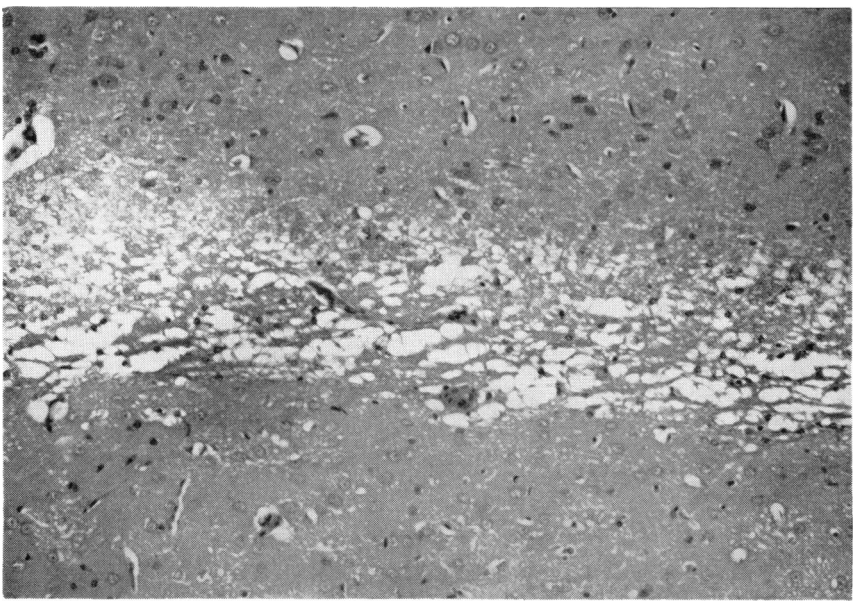

Fig. 19. Severe vacuolization of callosal radiation between cortex (above) and basal ganglion (below) following implantation of cryptococcal capsular polysaccharide. Hematoxylin-eosin stain; x 320.

In 1963, Aleu, Katzman and Terry[20] reported additional and somewhat surprising findings in the fine structure of the rabbit's brain during alkyl tin intoxication. Up to this point the issue was drawn between the light microscopists who supported the concept of interstitial edema, and the electron microscopists who believed that the excess fluid localized within the clear glial cells. Aleu and his co-workers injected the triethyl tin intraperitoneally and caused thereby severe cerebral swelling. They found predominantly a white matter lesion which consisted of fluid accumulation almost exclusively in large clefts within the myelin sheaths. The swelling of the clear glial cells in their rabbits was both mild and focal. They, too, failed to find an enlarged intercellular space. The vascular walls were intact and the blood-brain barrier to trypan blue, as in all previous experiments with alkyl tin intoxication, was not altered. Shortly after this report appeared, a further communication by Katzman, Aleu and Wilson was published which confirmed on chemical grounds their earlier electron microscopic findings. Following the intraperitoneal injection of triethyl tin

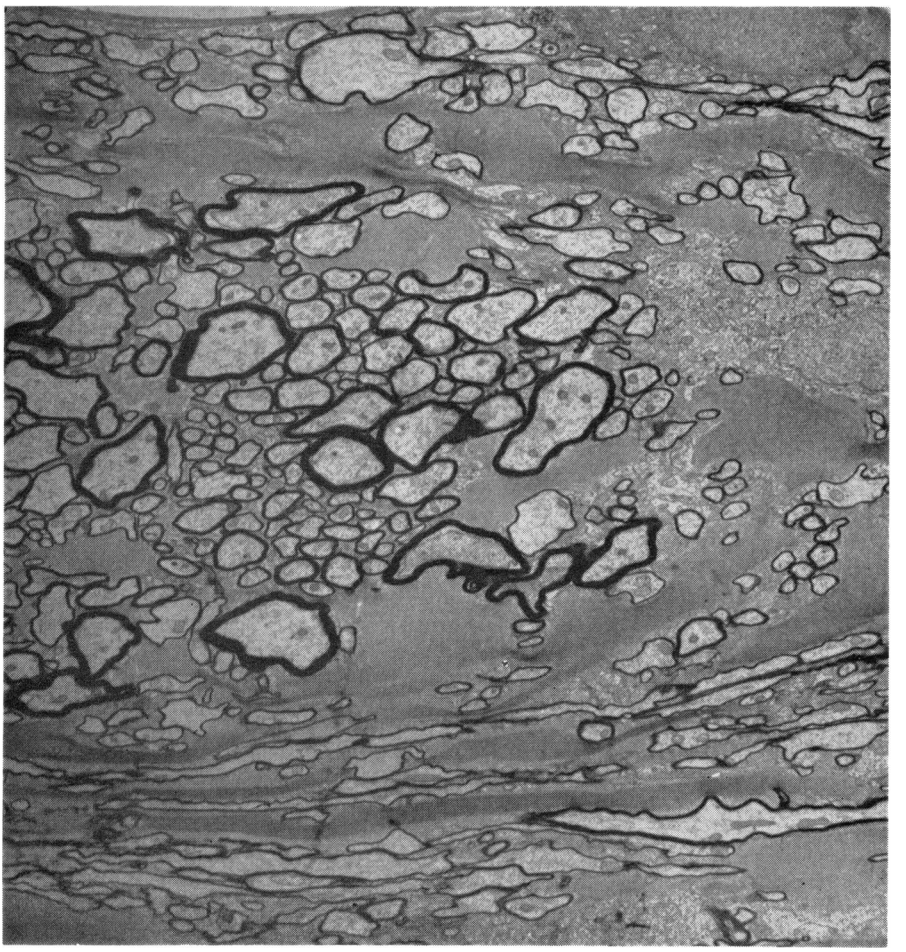

Fig. 20. Low-power electron micrograph to show irregular separation of neural tissue by electron-dense fluid in callosal radiation. Stained with uranyl acetate solution; x 4,000.

in rabbits, the water content of the white matter increased by 91 per cent compared to the dry weight. The four-hour S^{35} sulfate space, a measure of the extracellular space, remained at the normal level of 2.6 per cent. The 24-hour albumin I^{131} space also remained at the normal level of less than 2 per cent. From these and other chemical findings the conclusion was reached that the excess fluid accumulation was not extracellular, but was loculated within intramyelinic spaces.

Evidence had been produced earlier by Evans, Tani and Raimondi[21]

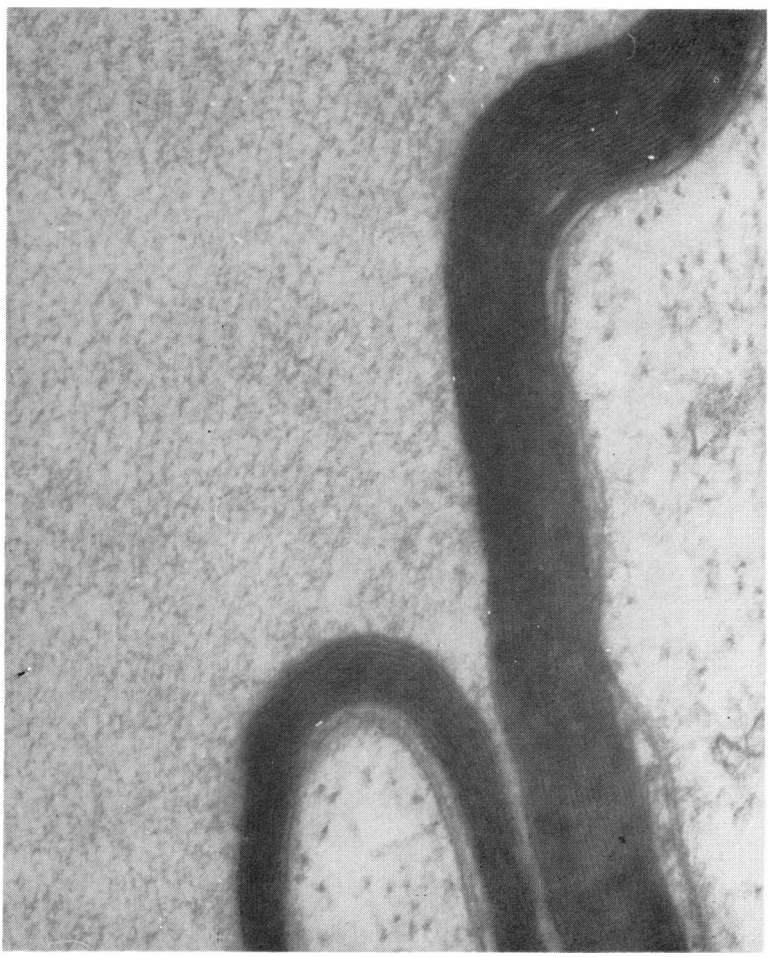

Fig. 21. Two adjacent myelin sheaths and surrounding electron-dense, reticulated fluid. There is no limiting membrane to demarcate the fluid, which is in contact with the outermost myelin lamellae. x 113,000.

that the swelling in the white matter caused by pressure on the brain exerted by an inflated extradural balloon was the result of separation of perivascular glial cells from the basement membrane of the vessels. This was proof that under the conditions of the experiment interstitial fluid accumulation did occur in perivascular regions.

It was apparent from all the previous experiments that the complete picture of intracerebral fluid accumulation was not yet at hand. Another study was undertaken at this point by my associates, Levine and

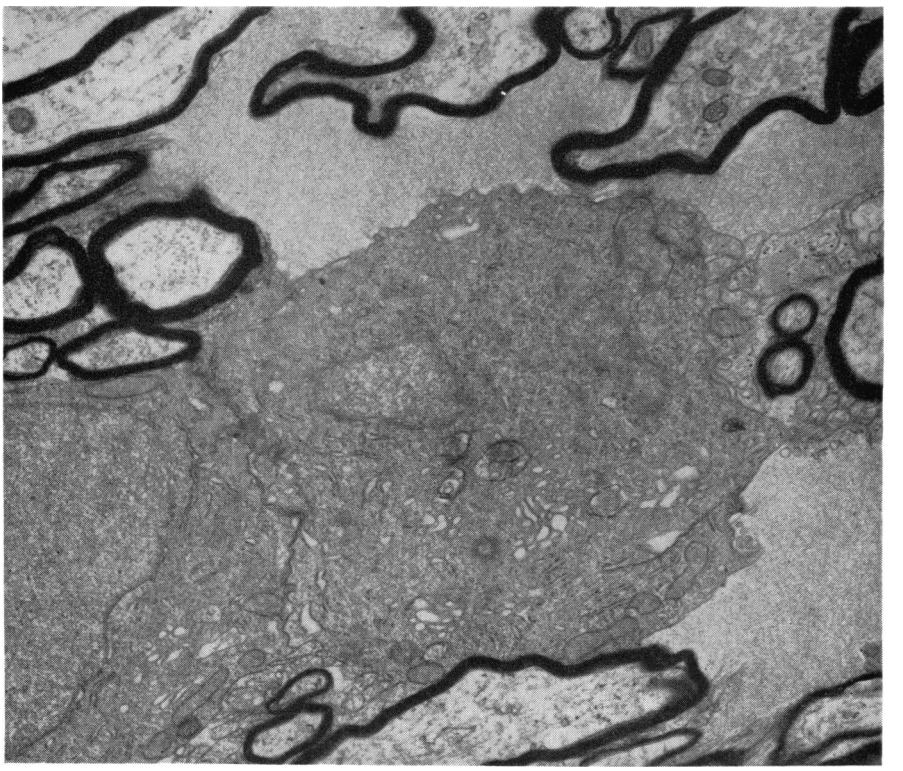

Fig. 22. Neuroglial cell and process reveal no fluid accumulation in spite of large extracellular pool. x 14,000.

Gonatas, for the purpose of clarifying the aspect of edema as part of the inflammatory response in the brain.[22,23] Utilizing the Krieg-Johnson stereotaxic instrument, various substances were implanted into the region of the callosal radiation of the rat's brain. Sterile graphite was used as the implant, then graphite with purified protein derivative of tuberculin (PPD), and pneumococcal and cryptococcal capsular polysaccharides. Certain inorganic and organic chemicals were also employed and produced local necrosis and widespread edema of white matter associated with increased permeability of the blood-brain barrier. In the acute phase of PPD-induced inflammation in the rat brain, the electron microscope revealed enlargement of the extracellular space in the white matter without, necessarily, any destruction of tissue by necrosis. Reactive clear glial cells, most probably related to the astrocyte, appeared and showed pinocytotic activity suggestive of fluid flow

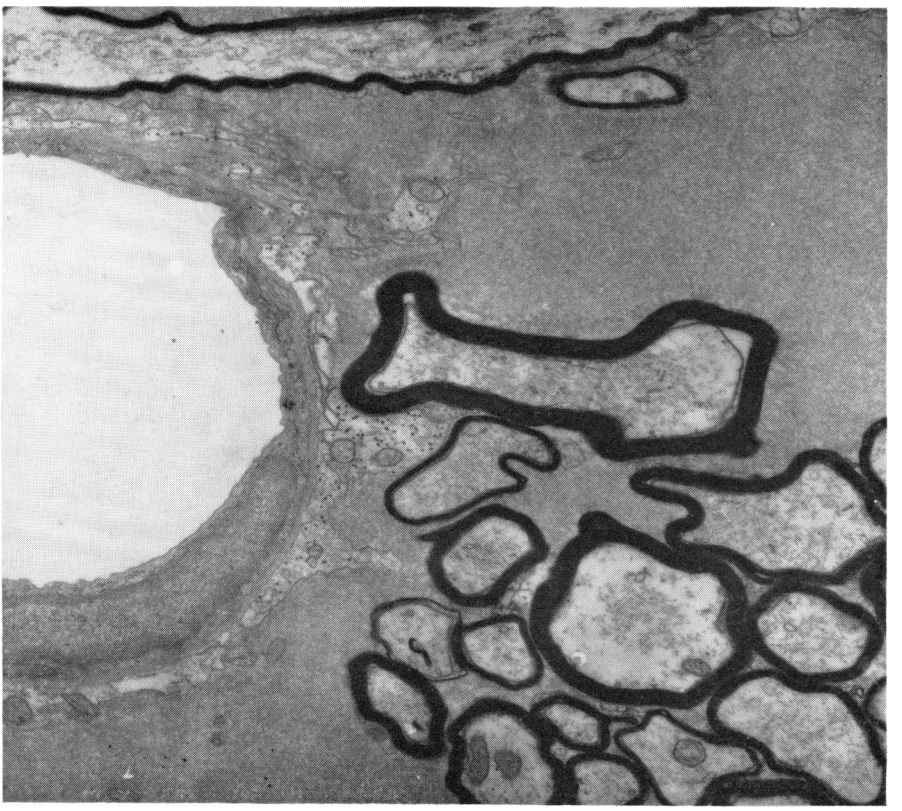

Fig. 23. Callosal radiation 48 hours after implantation of polysaccharide. Astrocytic vascular feet surround basement membrane, separating intercellular fluid from vessel wall. x 15,000.

from the extracellular space into the cell. There was also evidence of phagocytosis of myelin, predominantly by the macrophage but also, occasionally, by the reactive clear glial cell. The fluid distribution in PPD-induced edema was studied by Katzman, Gonatas and Levine.[24] These investigators found that the Geigy blue-stained areas of gray and white matter, which represent the sites of edema and breakdown of the blood-brain barrier, showed a greater accumulation of sodium ion and a more rapid uptake of $S^{35}O_4$ and albumin I^{131} than the unstained areas and the gray and white matter of the brains of control animals. In comparing these findings in PPD-induced edema with other forms of edema, they concluded that the edematous white matter was correlated with the development of a true extracellular space. In ede-

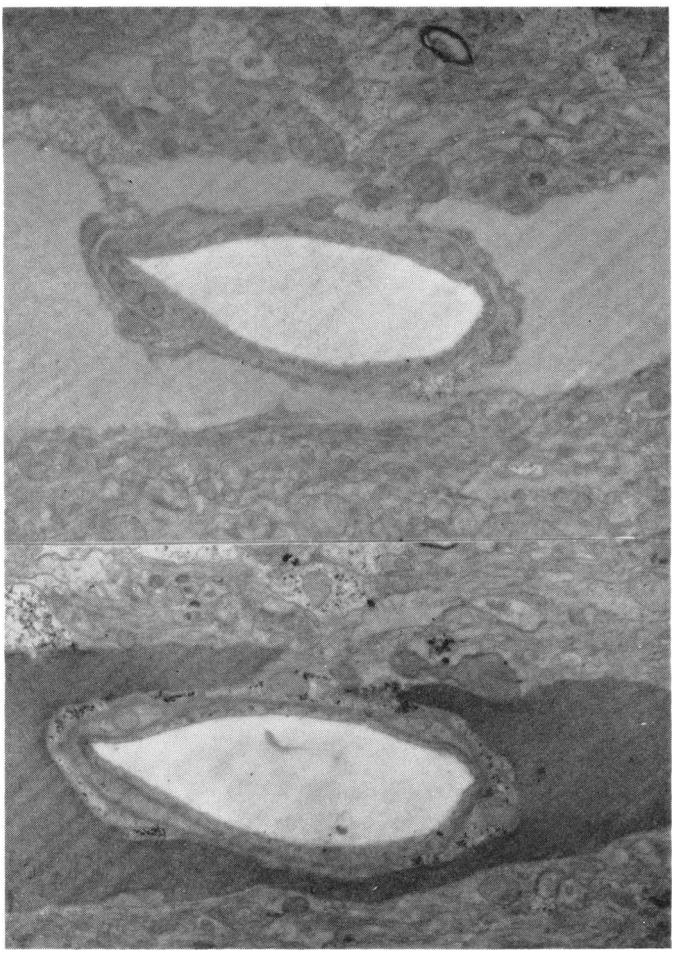

Fig. 24A

Fig. 24B

Fig. 24A and B. Fluid accumulation containing cryptococcal polysaccharide around a small vessel in cerebral tissue near the implant twelve hours after implantation. Compare density of fluid before and after staining with uranyl acetate. x 7,000.

matous gray matter, however, the only ultrastructural change observed was a swelling of the clear glial cells.

More recently Hirano, Levine and I followed the course of spread in the extracellular space of the cryptococcal polysaccharide during the acute stage.[25] Following the implantation of the capsular polysaccharide in the anterior end of the callosal radiation of the rat brain, there appeared severe vacuolation of the white matter in light microscopic preparations (Figure 19). Under the electron microscope, by

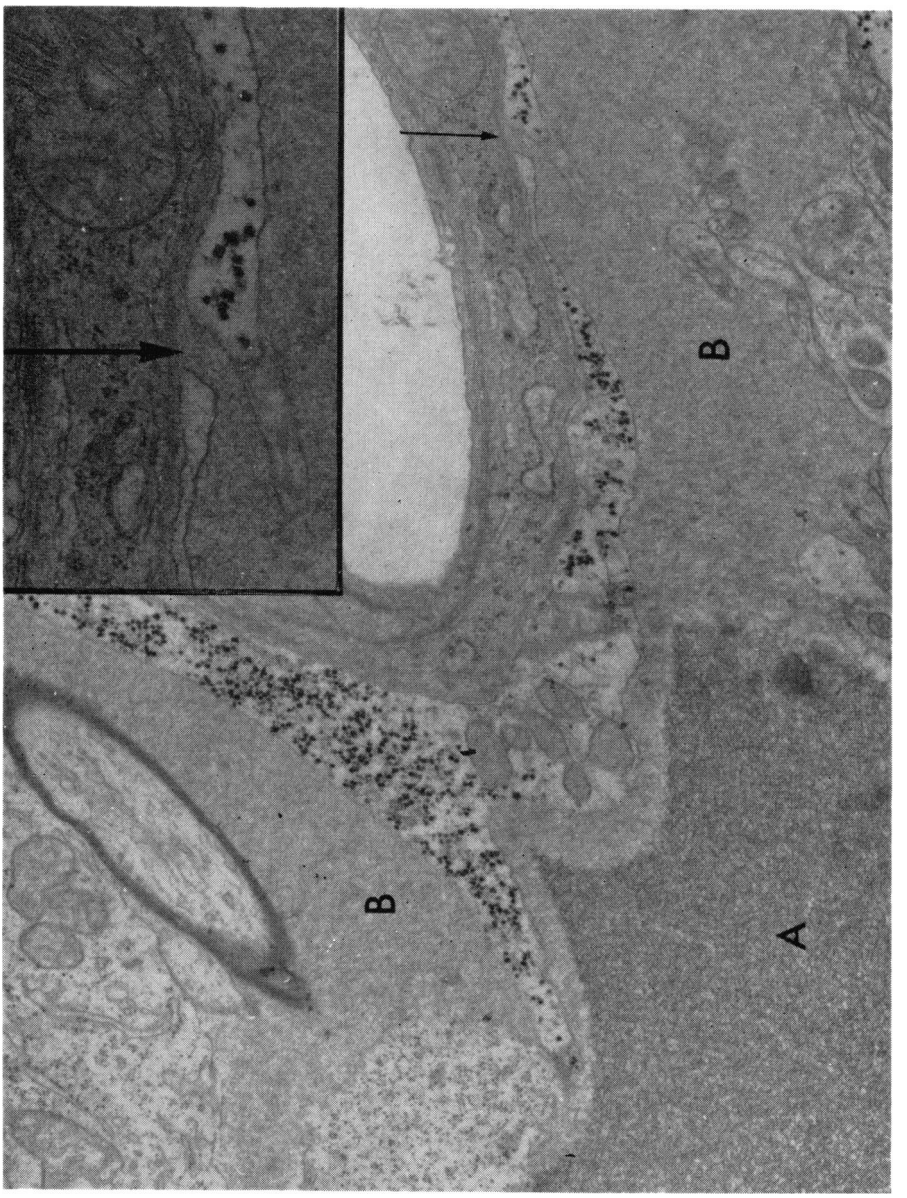

Fig. 25. Pools of extracellular fluid adjacent to small vessel near implant site 24 hours previously. A. Polysaccharide-containing fluid. B. Plasma-like fluid. Arrow points to gap in perivascular ring of astrocytic feet. Extracellular fluid communicates with material in basement membrane of vessel wall. Stained with lead hydroxide; x 22,000. Insert x 46,000.

contrast, Dr. Hirano found no optically empty spaces except for the lumens of vessels distended by the perfusion of the fixative materials. The cerebral tissue elements were irregularly separated by fluid (Figure 20). The myelinated nerve fibers were separated either as groups or individually by this electron-dense fluid. In all portions of the callosal radiation, and at all the intervals of time covered in this study of the acute phase, most of the fluid was located in the extracellular space (Figure 21). It was in contact with the outermost layer of the myelinated nerve fibers or with the plasma membrane of the glial or neuronal cell processes. There was no splitting or separation of the myelin lamellae to form intramyelinic cysts for the harboring of fluid. The neuroglial bodies and processes in the white matter revealed no accumulation of fluid within their cytoplasm (Figure 22). Individual cells were often widely separated from each other by large pools of fluid. Astrocytic foot processes kept their normal anatomic relationship to the vascular basement membrane; separation of the perivascular astrocyte from the basement membrane of the blood vessel was not observed (Figure 23). Preservation of the relationship of endothelial cell, basement membrane and astrocytic foot process was considered to be important because the lesion under study had exhibited little alteration of vascular permeability. Extracellular fluid in the cortical neuropil was usually limited to the junction area between cortex and subcortical white matter; it was not seen in superficial layers of the cortex. The opposite cerebral hemisphere, including the corpus callosum, was entirely normal.

Certain of these findings were difficult to interpret, the most important of these being the source of the intercellular fluid in the absence of an anatomic alteration in the blood vessel wall and its attached glial processes. The problem was soon clarified, however, upon further study.[26] Electron micrography revealed that the fluid resulting from polysaccharide implantation in the corpus callosum was not homogeneous, but consisted of two types. One, which contained a reticulated electron-dense substance, was found to contain the actual implanted polysaccharide; the other, of less dense appearance, was derived from the blood vessels at the site of implantation. At this site, the cryptococcal polysaccharide could be identified by the presence in the edema fluid of a uniformly distributed reticular substance that stained strongly with uranyl acetate (Figures 24A and B). The nonreticulated less dense fluid resembled blood plasma. The two types of fluid differed

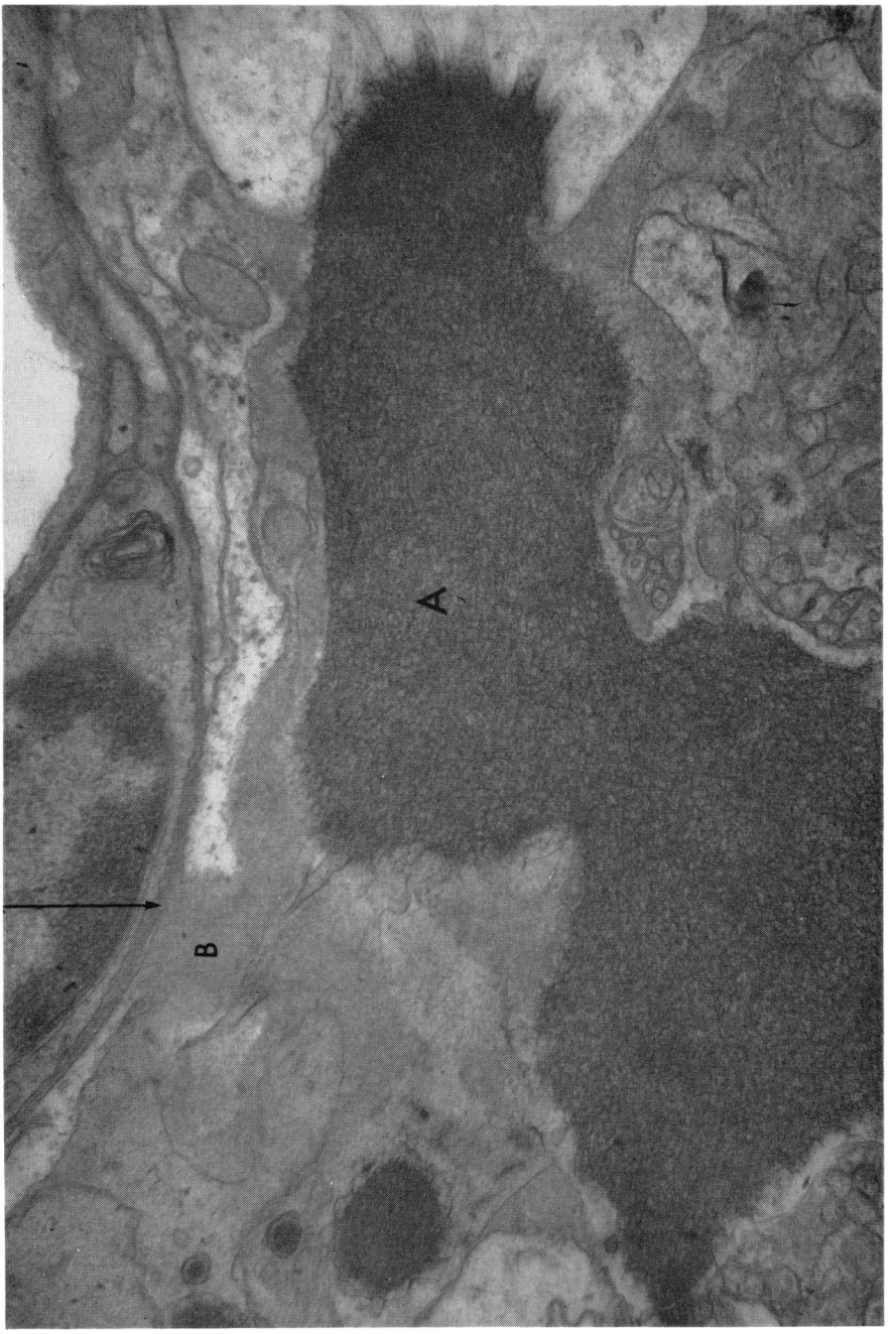

Fig. 26. Intercellular fluid near the margin of a cryptococcal polysaccharide implant. Electron-dense fluid (A) is surrounded by less electron-dense fluid (B). The latter is connected with material in the vascular basement membrane (arrow). x 24,000.

in location as well as in appearance. When together, the polysaccharide-containing fluid was always in the center and the plasma-like fluid was located between the former and the tissue cells. The plasma-like fluid occurred in the vicinity of blood vessels near the site of implantation. In these regions, separation of perivascular astrocytic foot processes occurred and plasma-like fluid communicated with the vascular basement membrane through gaps between foot processes (Figure 25). Disruption of the astrocytic basement membrane permitted continuity between the plasma-like fluid and the basement membrane (Figure 26). These changes were observed only in regions close to the implant and only during the early stages of the reaction. They were not seen in the distal portions of the callosal radiation. The differentiation of the two types of fluid was clear between 6 and 12 hours after implantation, was less clear after 24 to 35 hours, and was absent after 48 hours. Evidently this amount of time was necessary for their complete miscibility.

From all the electron microscopic studies on abnormal fluid accumulations in the brain we have learned a number of facts. One is that different types of injury can produce different responses as regards fluid localization. In the cortex this is manifested by swelling of the clear glial cells with no increase in the intercellular space. Certain injuries to white matter are characterized by intramyelinic splitting with fluid accumulation in the cystic spaces thus created. Other injuries result in vast collections of extracellular fluid sometimes with, but often without, simultaneous swelling of the glia. Thus it would appear that there is an ultramicroscopic difference between cerebral swelling and edema. Confirmation of this is yet to be made in man.

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